

Final Report

**Pilot Study to Develop Technical Standards and Procedures for Stream
Reference Reach Establishment in Virginia's Coastal Zone**



Submitted by:

Dr. Stephen P. McIninch
Dr. Greg C. Garman

Center for Environmental Studies and
Department of Biology
Virginia Commonwealth University
Richmond, Virginia 23284-2012

September 2002

Proper Citation: McIninch, S.P. and G.C. Garman. 2002. Pilot Study to Develop Technical Standards and Procedures for Stream Reference Reach Establishment in Virginia's Coastal Zone. Final Project Report to Virginia Department of Conservation & Recreation, Richmond, VA.

Funding for this report was provided through a grant from Virginia Department of Conservation and Recreation in coordination with Virginia Coastal Resources Management Program at the Department of Environmental Quality through Grant # NA07OZ136 of the National Oceanic and Atmospheric Administration, Office of Coastal Resource Management, under the Coastal Zone Management Act of 1972, as amended.

The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies.

Introduction

A key role of state and federal natural resources agencies is the identification and restoration of streams and riparian corridors that have been degraded by anthropogenic stressors, such as hydromodification and nonpoint source (NPS) pollution. Challenges associated with the restoration process include: 1.) development and application of appropriate stream assessment protocols and 2.) defining a set of measurable and diagnostic stream conditions as target endpoints for the restoration effort. Both of these challenges are dependent on an understanding of, and comparison to, relevant reference conditions that describe accurately the ecological potential of streams within a specific region. Reference streams provide, therefore, the blueprint for setting stream restoration goals and designing tactics to achieve those goals, as well as being objective and relevant criteria against which to judge the success or failure of stream restoration activities. The use of reference reaches as a management tool for assessment, maintenance, and restoration of watersheds has been adopted widely by local, state, and federal agencies within the mid-Atlantic region, including North Carolina, Maryland, Delaware, Pennsylvania, and Virginia.

Generally, stream reference conditions for a region are based on empirical data from relatively undisturbed streams. However, as a result of widespread development, many regions no longer support appropriate reference streams. In Virginia, this lack of relatively undisturbed ('pristine') streams is especially problematic in the Coastal Zone, northern Virginia, and the Shenandoah Valley and may compromise stream assessment and protection activities for these areas. Researchers elsewhere have proposed the development of objective and statistically valid model (i.e., *virtual*) reference streams and at least one state agency (New York DEP) has adopted these stream models into the regulatory process. In addition, stream restoration goals have traditionally focused on reestablishing 'natural' hydromorphological conditions (e.g. Rosgen classification) to degraded stream reaches, based on the assumption that the biological and ecological components will, in time, recover if the physical habitat is improved. This approach may ignore other important factors, including community structure and function, the timing of recovery, stream physicochemistry, and land-use within the watershed. Previous work by Virginia Commonwealth University (VCU) (Polecat Creek Watershed Investigation; Smock & Garman 2001) and others (Maryland Biological Stream Survey; MD DNR 2001) suggests that temporally and spatially synoptic results from dissimilar assessment protocols (e.g. instream habitat *vs.* macroinvertebrate community) are rarely correlated. Hence, hydromorphological criteria alone, or criteria based on any narrowly defined set of stream attributes, may not be useful stream restoration targets or evaluation criteria for stream assessments.

A variety of stream assessment protocols, both hydromorphological and biotic, have been applied widely within North America (e.g. Rosgen Stream Classification, EPA Rapid Habitat Assessment, EPA Rapid Bioassessment Protocols). However, these multimetric indices (RBP III, IBI) have been developed and tested primarily in moderate and high gradient streams outside of coastal areas, and may be unsuitable for accurately assessing coastal stream systems. Recently, researchers have attempted to modify selected protocols, such as those for benthic invertebrates, to more accurately apply to coastal streams (Maxted, et al. 1998; Smock and Garman 2001). However, no published studies have attempted to incorporate a wide range of stream conditions, including stream morphology, mesohabitat, and fish and benthic invertebrates, into a quantitative and statistically valid model of stream reference conditions.

The primary objective of this pilot study (Phase I) was to develop a draft, *virtual* reference stream model for several representative, upper Coastal Plain and Fall Zone watersheds, using multivariate statistical analyses to integrate data for over 35 ecologically relevant metrics from approximately 40 stream reaches. Using a large number of sites to develop ‘virtual’ reference conditions has several advantages both scientifically and from a management viewpoint: 1) sites, rather than multiple collections within sites, serve as valid statistical replicates (e.g. avoids problems of pseudo-replication), 2) allows for more robust variance evaluations than a single or limited number of reference sites would allow, 3) provides a greater capacity for extrapolation than site specific reference conditions, and 4) avoids problems associated with maintaining and monitoring specific control sites which may not retain their reference condition over time.

We targeted several small Coastal Zone watersheds to assess how best to combine, modify, or re-create the range of existing stream assessment tools (e.g. RBP, IBI) for use in establishing reference conditions using multiple sites within a region. One secondary objective of this pilot study was to evaluate the utility and validity of established stream assessment tools for discriminating natural, or minimally impacted sites, from impaired reaches of coastal streams. Modifications to these standard assessment methods to better address the unique attributes of coastal streams were also developed and evaluated statistically. Finally, those tools that combined most accurately distinguish regional reference conditions, based on both biological and physical integrity, were identified from a pilot data set.

Based on the findings of this pilot study, we propose to develop (Phases II and III) a suite of quantitative stream assessment tools, and a process for implementing those tools, for the Virginia Coastal Zone and additional regions. These assessment tools will facilitate stream classification, establish regional stream reference conditions (i.e., target conditions for restoration projects), and provide criteria for post-project evaluation. These assessment tools will benefit a wide range of stakeholders, including state agencies charged with implementation and regulation of stream restoration projects, regional and local governments responsible for developing watershed management plans that include monitoring and restoration goals, and non-governmental organizations such as volunteer watershed groups interested in planning and participating in stream restoration projects. These phased projects will support implementation of DCR’s NPS Pollution Control Program by addressing specific objectives set forth in Virginia’s NPS Pollution Management Plan.

Methods

Study Areas

The watersheds selected for this pilot study: Dragon Run, Polecat Creek, Totopotomoy Creek, and Upham Brook, are representative of the full range of variation in stream size and degree of impact, from relatively undisturbed to highly degraded. Dragon Run is a lowland watershed of the Piankatank River system found completely within the Coastal Plain physiographic province. It is bordered by extensive swamp, marsh, and forested wetland throughout much of its path. The remaining watersheds have some connection to the Fall Zone or lower Piedmont province. Upham Brook is a highly urbanized watershed within the city of Richmond and surrounding suburban areas. Totopotomoy Creek flows through developments in the Richmond suburban area of Hanover County and Polecat Creek watershed drains mostly agricultural land north of Richmond that is dominated by residential land use.

Historical data collected by VCU and VDGIF personnel were gleaned for data from the selected study watersheds. Because of ongoing studies in the Polecat and Upham Brook watersheds, there are significant amounts of recent biological and physical habitat data. Dragon Run and Totopotomoy Creek watersheds were in need of biological data collection and physical habitat analyses. All watersheds required Rosgen-type hydrogeomorphic analysis.

Specific study sites were randomly selected by ArcView GIS software in a two-stage process. First computer generated dots were randomly placed on stream segments corresponding to the mid-point of a 100-meter stream segment. Twice the number of sites required was selected in case some needed to be eliminated as inaccessible. The initial 100 sites were given unique numbers. The second stage of the selection process included the generation of a random numbers list and subsequent selection of sites corresponding to the numbers. Final site selection was made following a site visit to determine accessibility of the site, obtain landowner permission, and acquire initial habitat notes. Data collected included hydrogeomorphic measurements (Rosgen), physical habitat characterization, fish community data, and aquatic macroinvertebrate community data.

Stream classification and instream habitat characterization were conducted using standard protocols: Rosgen (1996) and EPA's rapid habitat assessment (Barbour et al. 1999), respectively. Selected 'Rosgen' metrics included: channel gradient, bank-full depth and width, entrenchment ratio (bank-full maximum depth/floodprone area width), stream width/depth ratio, and sinuosity.

The fish community was examined using standard backpack electrofishing protocols for coastal habitats (McIninch and Garman 1999; Smock and Garman 2001). Because all chosen study sites were wadeable only backpack electrofishing units were required for complete characterization of the fish community. Fishes were stunned working in an upstream direction for the entire 100-meter length of the study site. All fish were collected and placed into a livewell. When the 100-m mark was reached electrofishing was ceased and effort (in seconds of electrofishing) recorded. Fishes were individually examined for physical anomalies, identified, enumerated and released.

The aquatic invertebrates were collected using D-frame dip-nets and protocols modified from Maxted et al. 1998. Dip nets were used to jab in various habitat types for the collection of invertebrates. Habitat types included wood (large or accumulation of smaller woody debris), bank margins (including root masses associated with undercut banks), and leaf litter accumulations or submerged macrophytes. Habitats were sampled in proportion to their abundance within the entire 100 m sampling site. Samples were preserved in alcohol dosed with Rose Bengal stain and returned to VCU for subsampling, sorting, and taxonomic identification. A subsample of 200 organisms was selected as the sample abundance. Organisms were identified to the lowest practicable taxonomic level, which was species for most groups.

Metrics

Habitat/ Physical Data

The quality of habitat for each study site was determined by visual assessment (Barbour et al. 1999; Barbour and Stribling 1991). A total of nine habitat parameters were used to score each site (Table 1): instream habitat availability, pool depth and variety, channel alteration, sediment deposition, channel flow, bank stability and vegetation characteristics, and condition of the riparian zone. Each metric was rated using

a numerical scoring system where 0 is the poorest condition and 20 points is awarded for the best (or optimal) condition. For bank vegetation and riparian zone metrics, each bank was rated separately (0-10 points) and aggregated to provide one score for the metric.

Table 1. Physical habitat parameters assessed/measured for pilot study (Phase I).

Habitat assessment

1. Amount of epifaunal substrate or available cover for aquatic organisms
2. Characterization of substrates associated with pool habitat
3. Variation of pool types within the study site
4. The extent of alteration, if any, to the stream channel
5. Extent of sediment deposition throughout the study site
6. Extent of water flow in the channel
7. Type and degree of bank vegetation
8. Stability of bank sediments
9. Width of the vegetated riparian zone

Rosgen stream classification

1. Percent slope of stream throughout study site
2. Sinuosity of stream (channel length over valley length)
3. Entrenchment ratio (width of floodprone area to width at bank-full height)
4. Ratio of stream width to depth

Fish and Macroinvertebrate Data

Twelve fish metrics were used to assess the fish community at every site. These metrics complement those of the Index of Biotic Integrity originally developed by Karr (1981) for Midwestern streams and modified for use in Virginia's coastal area by the authors (McIninch and Garman 1999). The IBI design is intended to assess the fish community through three groups of metrics corresponding to diversity and abundance of the fishes, functional composition and overall health of the fish community. The twelve metrics used for this pilot study and a brief description of their measurement is presented as Table 2.

Table 2. IBI Metrics used for Freshwater (Coastal Plain and lower Piedmont)

Metric 1 -- Species Richness.

Total number of **native** species in the sample, not including hybrids. Because of their long freshwater resident status (up to 20 year; Jenkins and Burkhead 1994), American eels are considered resident species. Introduced species are considered elsewhere (Metric 11).

Metric 2 -- Total Number of Individuals

The total number of individuals in the sample, expressed as catch per unit effort (CPUE), where effort is backpack electrofishing time (minutes).

Metric 3 -- Total Number of Darter Species

The total number of darter (*Etheostoma* and *Percina* spp.) species per sample. A total number of 6 species is possible.

Metric 4 -- Total Number of Sunfish Species

The total number of members of the Centrarchidae family, black basses included.

Metric 5 -- Total Number of Sucker Species

The total number of sucker species (family Catostomidae) in the sample;

Metric 6 -- Intolerant Species

The total number of species, per sample, classified as “intolerant” of degraded stream conditions. Intolerant species will include northern hogsucker, rosyside dace, stripeback darter, shield darter, and least brook lamprey.

Metric 7 -- Tolerant Species

The percentage of individuals classified as “tolerant” of degraded stream conditions. This metric will use the relative abundance of a guild of species to replace the “green sunfish” metric of Karr (1981), as suggested by Karr et al. (1986). Tolerant species will include creek chubsucker, American eel, golden shiner, pumpkinseed, bluegill, common carp, goldfish, brown bullhead, eastern mudminnow and tessellated darter.

Table 2. IBI Metrics used for Freshwater (Coastal Plain and lower Piedmont)

Metric 8 -- Omnivorous species

The percentage of individuals per sample classified as omnivores; species will include: creek chubsucker, goldfish, common carp, chubs of the genus *Nocomis*, spottail shiner, brown bullhead, white sucker, white catfish and channel catfish.

Metric 9 -- Insectivorous cyprinids

The percentage of cyprinid individuals per sample classified as insectivores; species will include satinfish shiner, swallowtail shiner, common shiner, comely shiner, rosyside dace, rosyface shiner, and blacknose dace.

Metric 10 -- Piscivores

The percentage of individuals per sample classified as facultative piscivores (apex predators), species will include: bowfin, longnose gar, chain pickerel, largemouth bass, black crappie, and blue catfish

Metric 11 -- Introduced species

The percentage of individuals per sample classified as non-indigenous species. This metric replaces the “hybrid” metric of Karr (1981) because hybrid identifications are often problematic especially in the field. Moreover, the numerical dominance of exotic taxa in disturbed ecosystems is well documented in the literature. Both the new “introduced” metric and the “hybrid” metric (Karr 1981) influence the overall IBI score most significantly under poor and fair stream conditions.

Metric 12 -- Anomalies

The percentage of individuals per sample exhibiting external parasites, infections, deformities, or skeletal anomalies. Minor blackspot found on individuals is not considered an anomaly.

Similar metrics assessing macroinvertebrate communities were also used at each study site. The seven metrics calculated have been determined as being the most appropriate to use in the coastal area (Smock and Garman 2000). The metrics used included richness measures, composition measures, tolerance measures, and habitat measures (Table 3).

Table 3. Rapid Bioassessment Protocol III Metrics used for Macroinvertebrate assessment. Scoring follows Smock and Garman (2001).

Metric 1 – Taxa Richness

The total number of taxa identified

Metric 2 – Modified Hilsenhoff Biotic Index (HBI)

$$HBI = \sum (X_i t_i / n)$$

where X_i = number of individuals of taxon i in a sample;

t_i = tolerance value of taxon i ;

n = total number of organisms in the sample

The HBI offers a quantitative assessment of the tolerance of each taxon to general water quality degradation. Tolerance values have been derived from Lenat (1993) and Pflakin et al. (1989). Values for rare species without published values were estimated based on experience of macroinvertebrate team.

Metric 3 – Scraper ratio

This is the direct ratio of the number of individuals in the scraper functional feeding group to those in the collector or filterer feeding groups.

Metric 4 – EPT ratio

This is the direct ratio of the individuals in insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddis flies) to the number of individuals in the dipteran family of Chironomidae.

Metric 5 – Percent dominant taxa

The percent contribution of the dominant taxon in the sample as the number of individuals of the most abundant taxon divided by the total number of individuals.

Metric 6 – EPT index

The number of individuals in the three orders listed above (EPT) divided by the total number of individuals.

Metric 7 – Percent shredder taxa

The number of individuals in the shredder functional feeding group divided by the total number of individuals.

Statistical Analyses

Data was assembled into a single database for analysis. We used Microsoft Excel, dBase 5.0, and the FORTRAN based CANOCO program. All raw data was initially examined through quality assurance procedures for data entry errors, outliers and those variables exhibiting no variation. Appropriate corrections and transformations were made to the data prior to further analysis. We used ASSESS Version 3.0 to compile index of biotic integrity scores for fish data and Rapid Bioassessment index scores for macroinvertebrate data. Correspondence analysis (CA) and detrended correspondence analysis (DCA) were used to arrange (ordinate) the study sites along axes based on the physical and biological data collected. The objective of ordination is to arrange the points such that points that are close together correspond to sites that are similar in those attributes examined (biological and/or physical data). Those sites most representative of undisturbed conditions would, therefore cluster together and be distant from those sites that represent heavily degraded conditions.

The initial set of ordination analyses also allowed for the reduction in number of variables by eliminating those variables that did not significantly contribute to the separation, or clustering, of sites. The elimination of certain variables is not an indication of their importance to the structure or function of the ecosystem, rather that the variability among all those sites examined was not strong enough to aid in the separation among sites. The initial CA was performed on the fish, macroinvertebrate, and physical data separately. Because of the large number of species and variables in general, the arch effect was present in many of the initial procedures. If the arch effect was noted from initial analyses (i.e. if sample scores on the second axis approximate a quadratic function of the scores on the first axes), then detrended correspondence analysis (DCA) was performed using a 2nd order polynomial detrending to remove this effect (Jongman et al. 1987; Ter Braak 1988). Once the insignificant variables were removed, remaining variables were lumped into a single file and analyzed again using DCA. The final direct gradient analysis was a canonical correspondence analysis where both biological and physical data are present. Again, the results are a graphical representation of the relations among sites based on the variables of interest. The gradients of the figure (graphical representation; left to right and top to bottom) are representations of how strong any given variable separates or clusters the sites. Further explanation will be presented in the results section.

We assumed that there was an underlying (or latent) structure in the data, i.e. that the occurrences of the biological communities and characteristics of the physical data are determined by some unknown set of parameters that reflect anthropogenic impacts, or lack thereof. In order to examine the response of our data to a simple model we performed multiple linear regression analyses (stepwise procedure, SPSS®) with selected variables from the previous analyses shown to be the strongest correlated with study site ordination. Variables that did not meet statistical assumptions (e.g. normality) were transformed as appropriate. All percentage data were arcsine transformed. The site scores (i.e. coefficients from the final DCCA) were entered as the response variable and significant biotic and abiotic variables entered as explanatory variables. Those statistically significant ($P > 0.05$) were used to develop the 'virtual' stream model. This model will be refined and built upon when additional data are available.

RESULTS

A total of 43 sites were examined for physical habitat characteristics, hydromorphic attributes, and the structure of both the fish and aquatic invertebrate communities. The majority of sites were located in the Polecat Creek watershed (22), York River drainage. Nine other sites were located from both Dragon Run and Totopotomoy watersheds (18) and the remaining three were urbanized sites in the Upham Brook system. A total of 111 macroinvertebrate taxa were identified during the study as well as 39 species of fish. Twelve IBI metrics and seven RBP III metrics were used to characterize the fish and macroinvertebrate assemblages, respectively (Tables 2 & 3).

Stage One – Exploratory Ordinations

The preliminary ordination analyses were performed on three separate databases, fish community data, macroinvertebrate community data and physical habitat/geomorphologic data. The resulting diagrams/ordinations are representations of site and species along two axes (Axes I and II). The ordination axes can be thought of as being theoretical environmental variables or underlying gradients in the data. For our purposes, we are attempting to view how the various study sites are aligned along this unknown gradient based on species data or the physical habitat data. The first round of analyses show that separation of sites varied and that no single dataset resulted in a clear separation of sites. At the very least we would expect the heavily urbanized streams of the Upham Brook watershed to separate from those less impacted streams of Dragon Run watershed.

Figures 1 and 2 represent the first two axes of the Detrended Correspondence Analysis (DCA) with the physical habitat and geomorphic data. Figure 1 shows the placement of the variables on the first two axes. Variables are abbreviated so that more labels may be placed on the figure. Those variables not visible on the figure are hidden because they are behind others. Figure 2 is the spread of sites on the first two axes. They are clustered based on their similarities and/or differences of the physical data. Note that there is little to no clear separation of sites. The first four axes of the DCA explained only about 40% of the variation in the data set.

Similar results are presented in Figures 3 (macroinvertebrate species) and 4 (site ordination based on macroinvertebrate species) and Figures 5 (fish species) and 6 (site ordination based on fishes). The first four axes of the macroinvertebrate DCA explained only 27% of the variation found in the data matrix. DCA based on fish species data explained about 53% of the variation. While stronger than the other datasets, we believe that without further analyses or different variables, neither the individual biotic communities nor the physical data are able to detect clear variation among the study sites chosen.

Figure 1. First two axes from Correspondence Analysis of abiotic habitat variables and geomorphology parameters. Symbols represent variables listed in Appendix II.

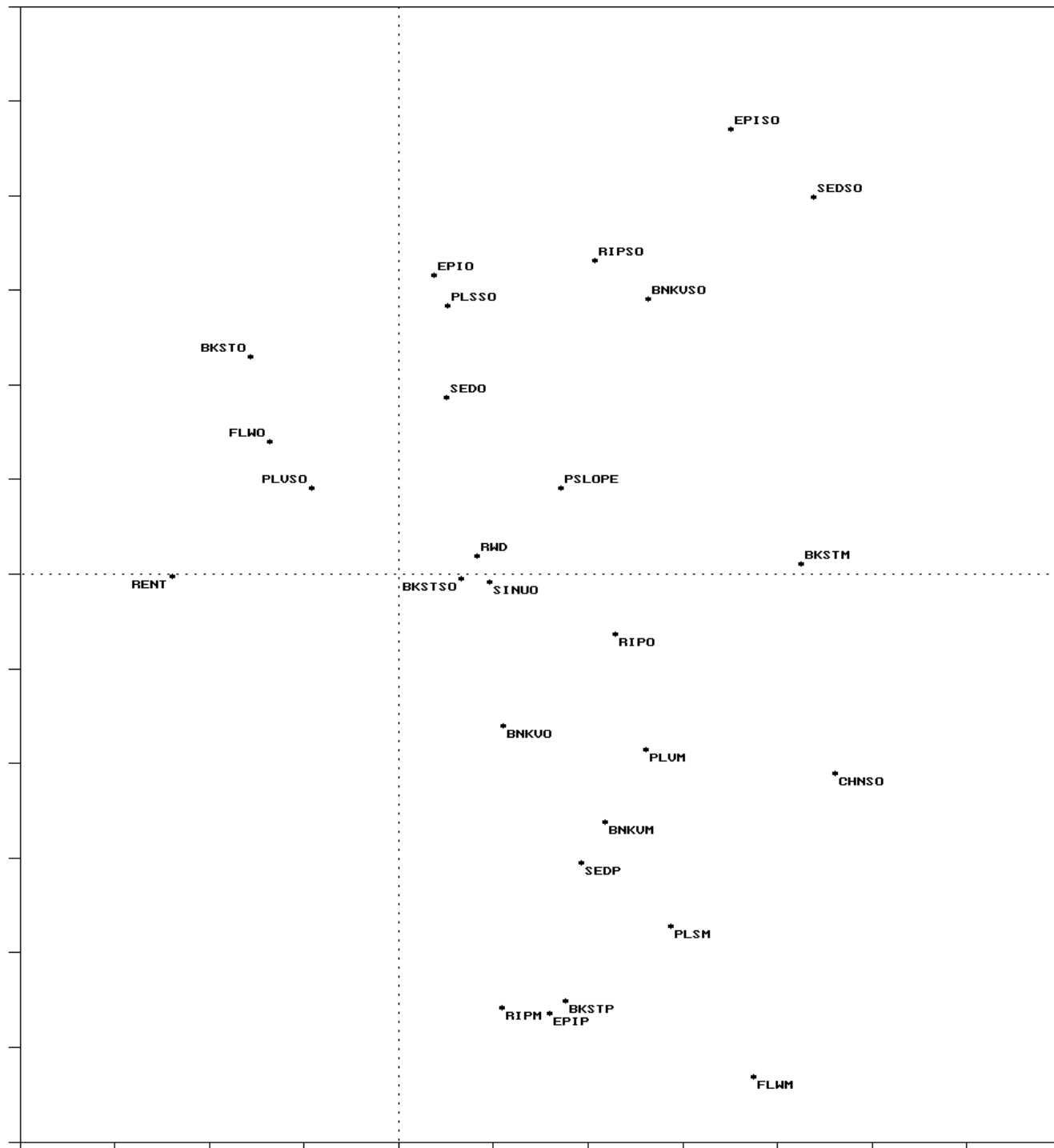


Figure 2. First two axes from Correspondence Analysis of abiotic habitat variables and geomorphology parameters. Symbols represent study sites.



Figure 3. First two axes from Correspondence Analysis of quantitative macroinvertebrate numbers and sites. Symbols represent study sites.

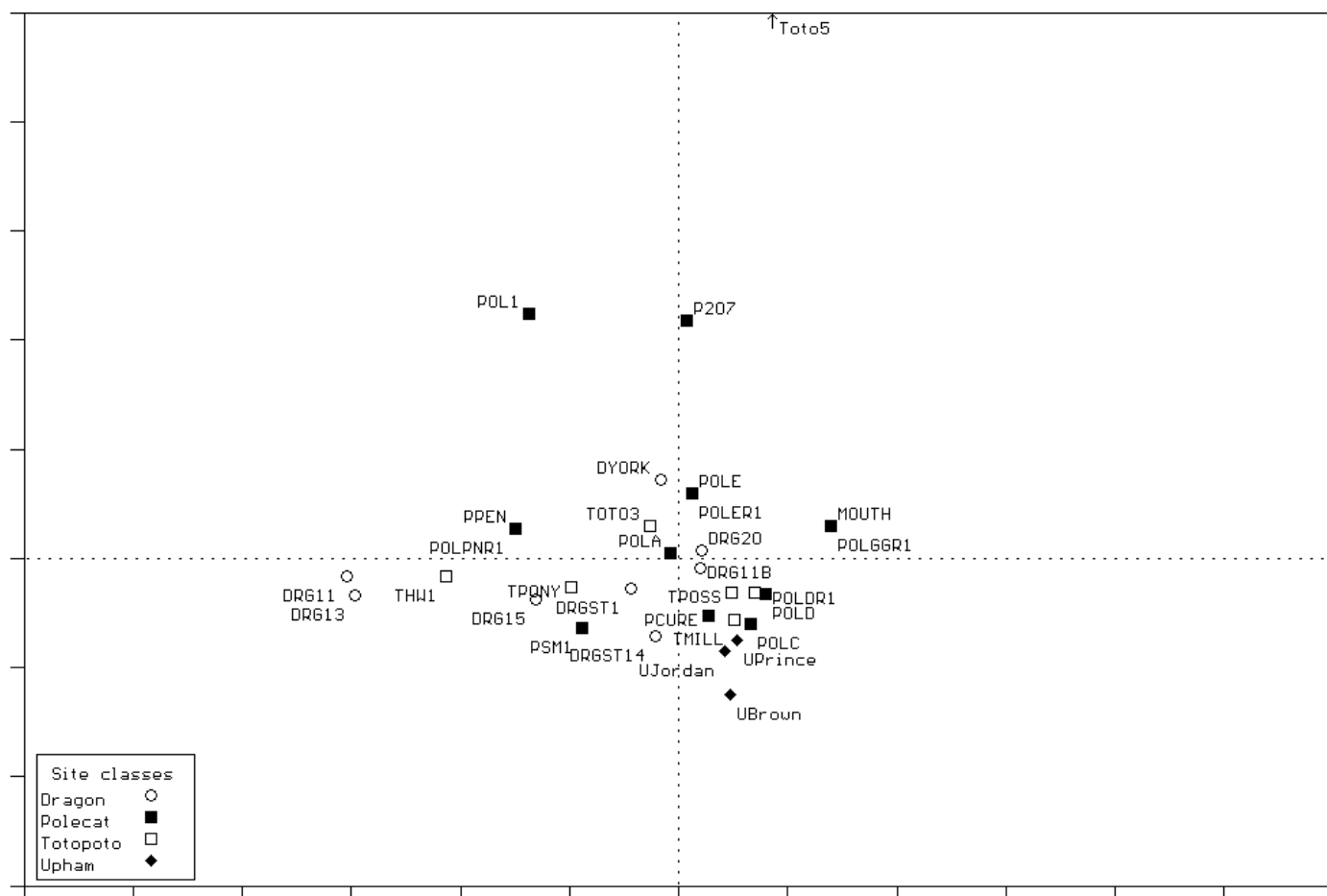


Figure 4. First two axes from Correspondence Analysis of quantitative macroinvertebrate numbers and sites. Symbols represent macroinvertebrate taxa listed in Appendix I.

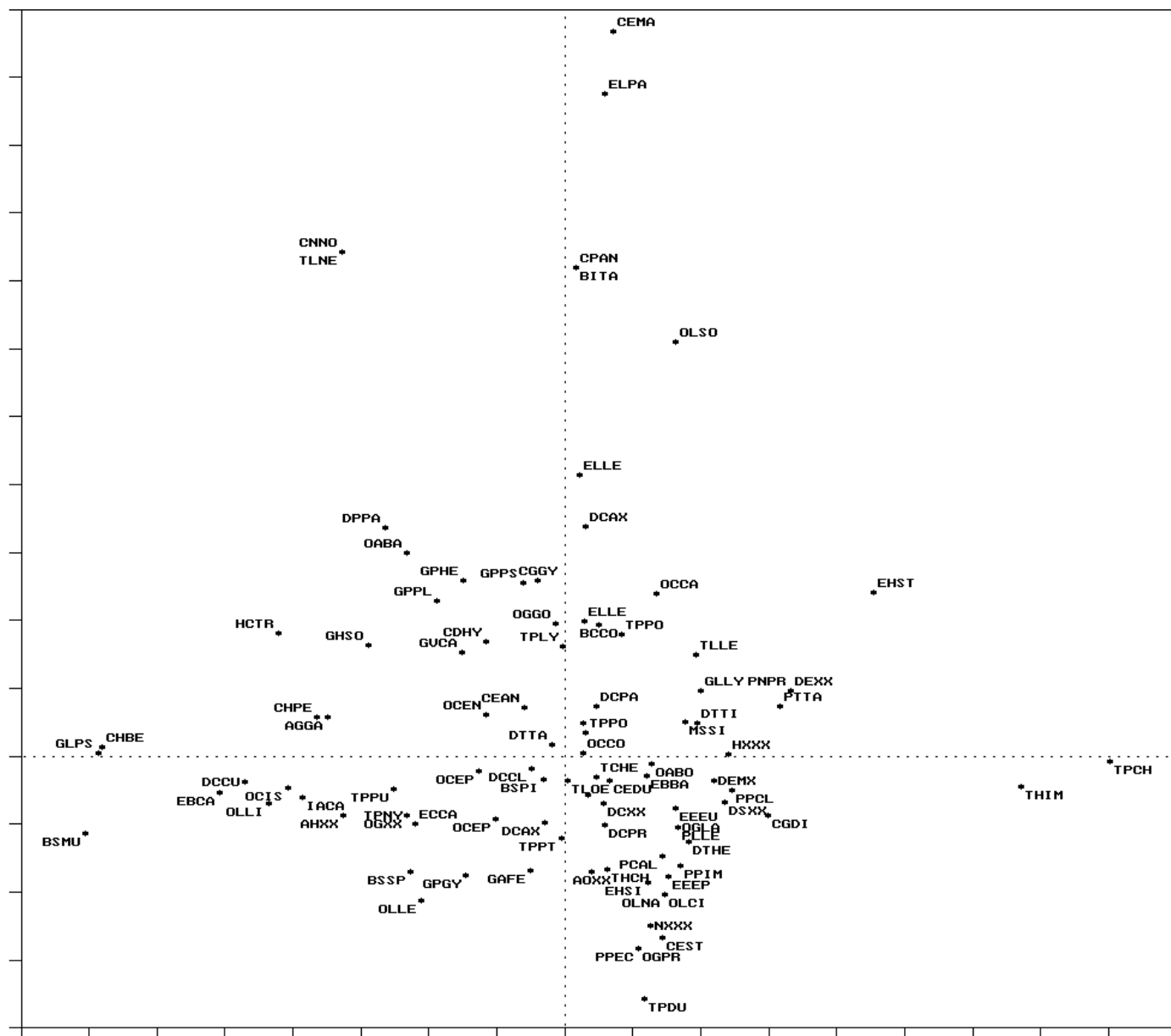


Figure 5. First two axes from Correspondence Analysis of quantitative fish numbers and sites. Symbols represent study sites.

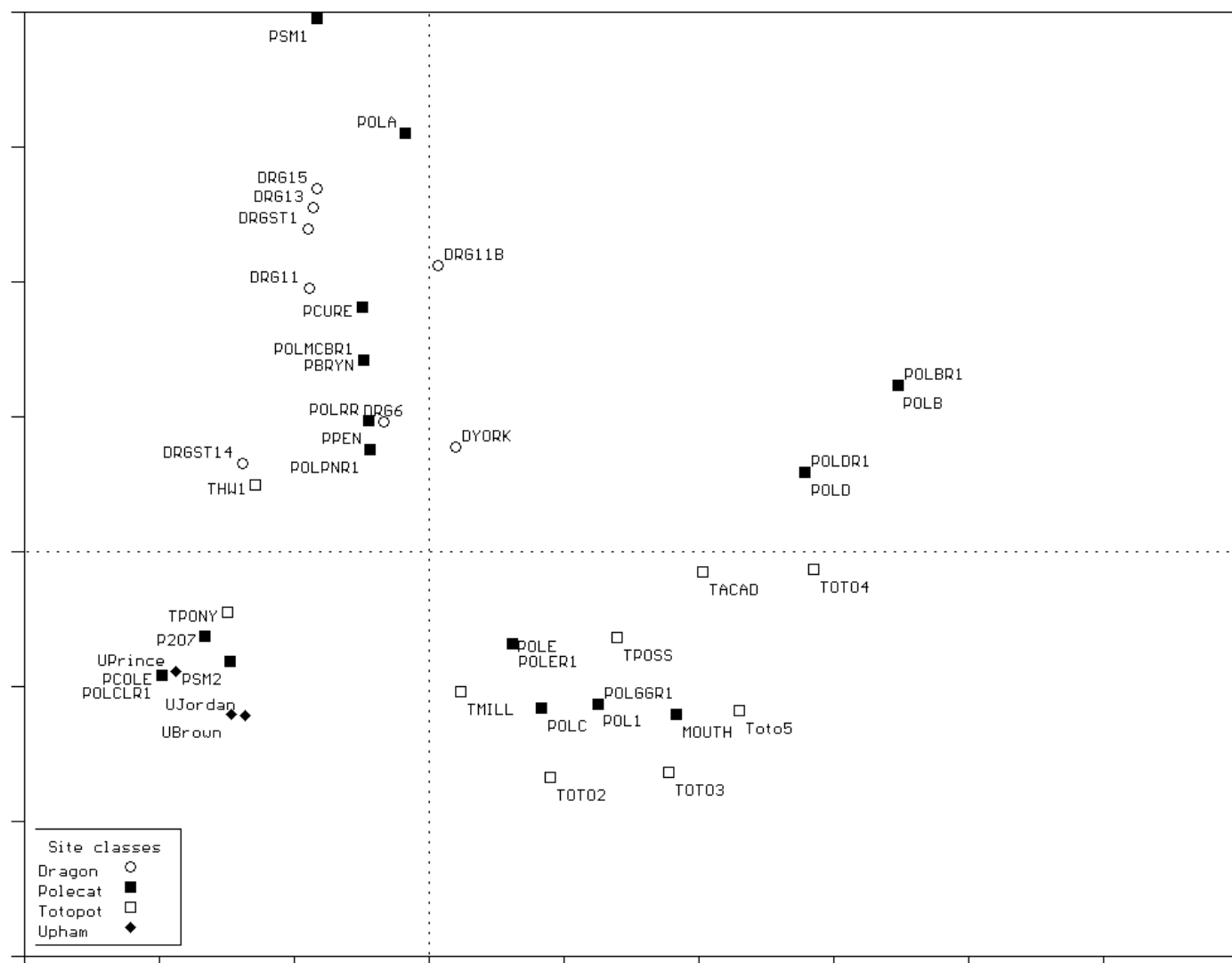
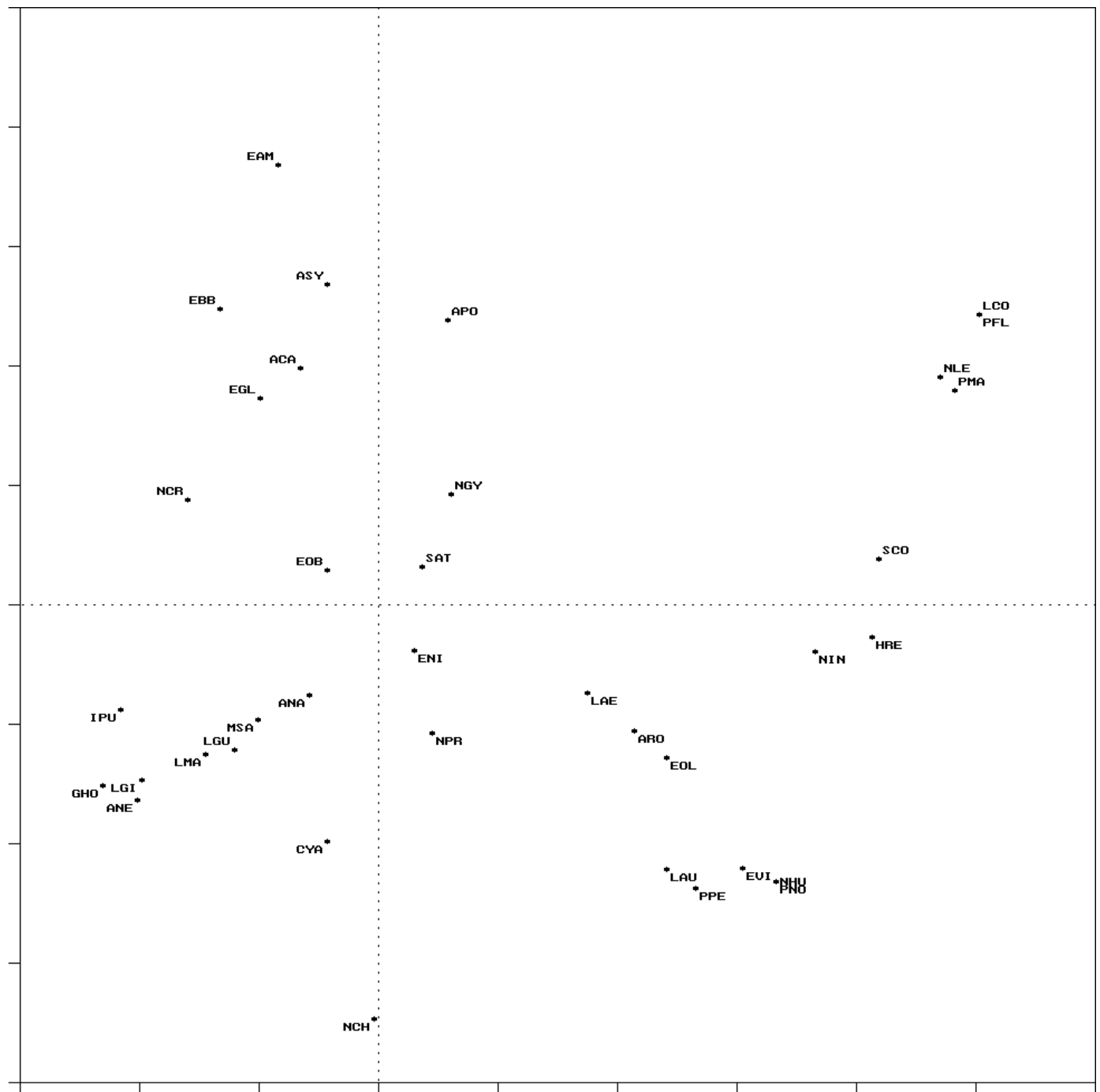


Figure 6. First two axes from Correspondence Analysis of quantitative fish numbers and sites. Symbols represent fish taxa listed in Appendix I.



Stage Two: Ordinations based on metrics of biotic community indices

The next series of analyses incorporated the Index of Biotic Integrity based on fishes and the Rapid Bioassessment Protocols based on macroinvertebrate species. The metrics of these indices were designed to better portray the structure and functional attributes of the respective biotic assemblages. A brief description of the metrics for both the IBI and RBP are found in Tables 2 and 3, respectively. We used the values of the metrics (not scores) to again ordinate our study sites. Figures 7 and 8 represent the spread of sites on the first two axes using the macroinvertebrate RBP metrics and the fish IBI metrics, respectively. Figure 7 shows a slightly better spread of sites in comparison to the stage one ordination based on macroinvertebrate species numbers. The greatest separation appears to be noted in the first axis (left to right in Figure 7). The gradient is largely based on the distribution of values for two RBP metrics; 1) ratio of the scraper functional feeding group to collector-filterers group (+ association with axis; right side of figure) and 2) ratio of numbers of individuals as members of the Ephemeroptera, Plecoptera and Trichoptera insect orders to members of the Chironomidae family (- association with axis).

Ordination based on the IBI metrics also exhibited some significant separation and spread of site data (Figure 8). Note in the right quadrants of Figure 8 that all three Upham sites (diamond symbols) are closely situated along the first axis (moving right to left). This suggests that the IBI metrics are able to differentiate some characteristics of the fish assemblage that is distinctive among the highly urbanized Upham Brook sites. It also suggests that those sites in close proximity (such as the Pony Swamp site in Totopotomoy watershed) share fish assemblage characteristics with the highly urbanized sites. The first axis (moving right to left in the Figure) represents a gradient of high numbers of anomalies in individuals and high percentage of introduced fishes (+ association; right side of figure) and higher percentages of piscivores, insectivores, and intolerant fishes on the left side of the graph (- association).

The upper portion of the second axis (top to bottom) is inhabited by sites that scored strongly for percent introduced species, percent piscivores, and percent fishes with anomalies. Sites associated with intolerant species, greater number of darter species and percent insectivorous fishes, inhabit the lower portion of the figure.

Figure 7. First two axes from Correspondence Analysis of metric values from Rapid Bioassessment Protocol and sites. Symbols represent study sites.

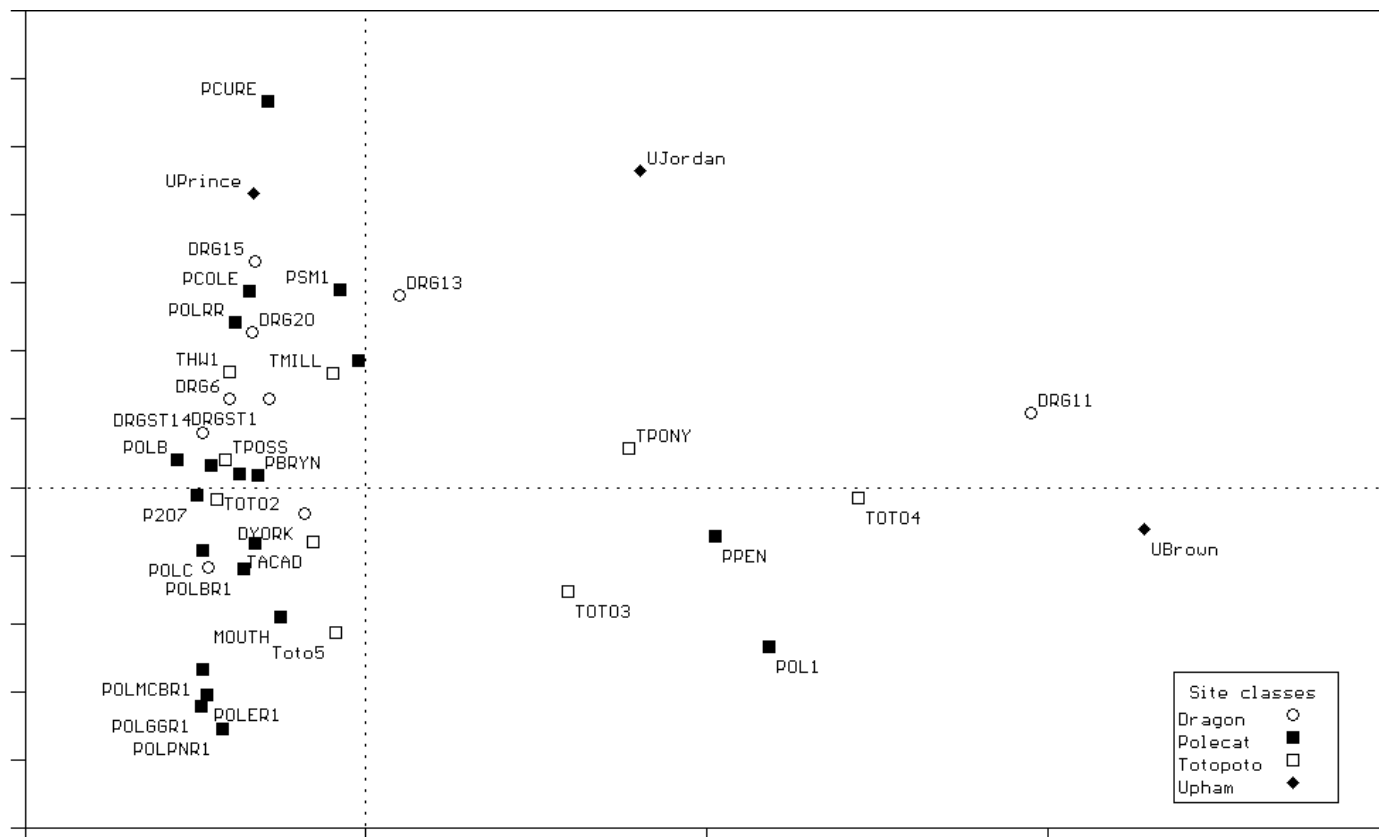
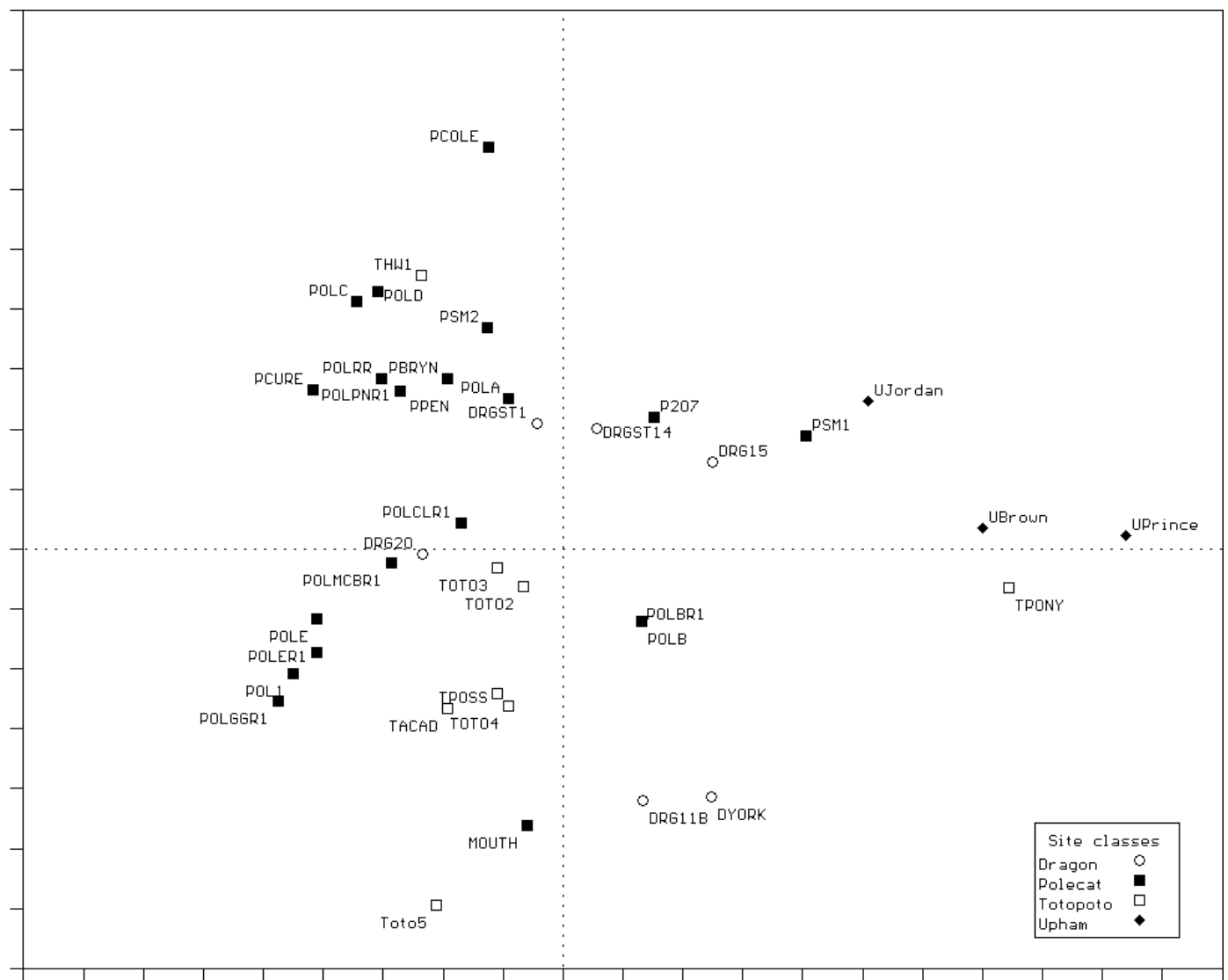


Figure 8. First two axes from Correspondence Analysis of metrics from Index of Biotic Integrity with fishes and sites. Symbols represent study sites



Stage Three: Data reduction and final analyses (DCA & DCCA)

The steps following the initial set of DCA procedures reduced the number of variables by examining the detailed results of the analysis and filtering out insignificant variables. Thus, any variable that did not contribute significantly to the fitting on any of the first four axes was automatically removed from further analyses. Following the first cut of variables, the remaining three datasets (physical data, fish metrics and macroinvertebrate metrics) were combined into a single working set. Further reduction in variables followed the next set of ordinations (DCA- run 2). Resultant statistical outputs were examined for the presence of correlation among variables. When two or more variables exhibited high inflation factors it was considered an indication of significant correlation and one of the variables was removed. The choice of which of two variables to remove was based on the overall contribution of each of the variables throughout the analyses.

The biotic metrics data (19 variables) were reduced to nine significant variables (Table 4). Similarly, the physical data was reduced to nine variables. The final DCCA included the biotic species data (fish and bugs) and the physical habitat variables in order to detect the main pattern in the relation between the species and the observed environment. Figure 9 represents the site ordination based on the biotic assemblage and the eighteen metrics and habitat variables found to be significant in previous analyses. The left side of the first axis is influenced by the HBI values from the macroinvertebrate data and the extent of introduced and tolerant species from the fish data. The sites found on the right side of Figure 9 would be more closely aligned with areas of high insectivorous fish populations, greater fish species richness and good quality epifaunal cover and riparian zones. The strongest variables associated with the second axis reflect the extent of stream entrenchment and the relative abundance of the shredder functional feeding group of the aquatic macroinvertebrates on the upper quadrants. The negative associations with the second axis were represented by the guild of insectivorous fishes. Table 5 is the listing of canonical coefficients for the final analysis indicating the strongest habitat variables and their relation to the first two axes.

Table 4. Eighteen physical variables and metrics parameters used for final reference reach model development.

Physical

1. Epifaunal Substrate
2. Extent of channel alteration
3. Extent of channel flow
4. Vegetative protection from bank
5. Stream bank stability
6. Entrenchment ratio
7. Percent stream slope
8. Ratio of stream width to depth
9. Width of riparian zone

Table 4. (cont.) Eighteen physical variables and metrics parameters used for final reference reach model development.

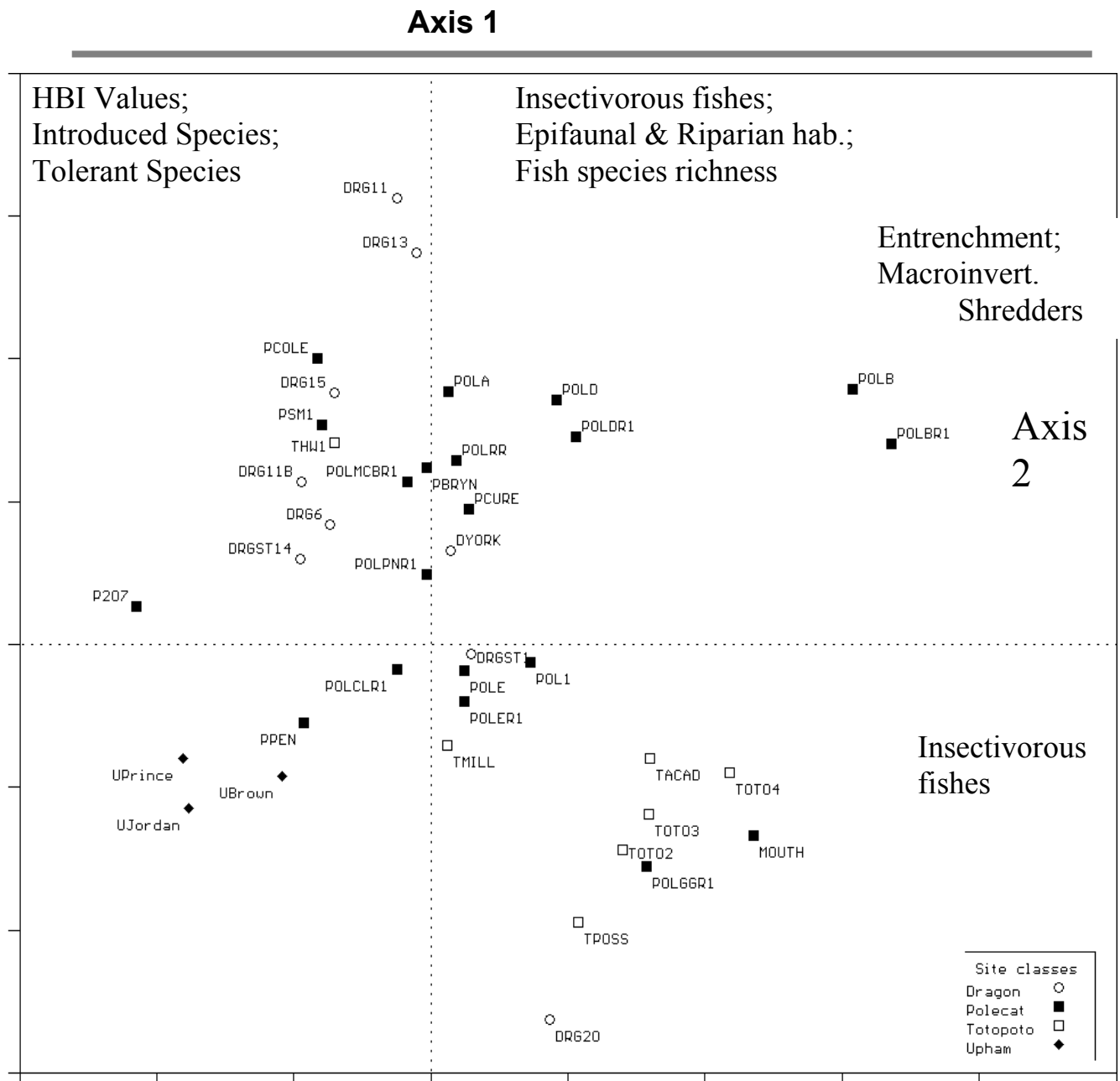
Biotic

1. HBI
2. Ratio Ephemeropterans, Plecopterans, and Trichopterans to Chrinomids
3. Ratio of shredders to all taxa
4. Fish species richness
5. Number of darter species present
6. Percent specimens as tolerant fishes
7. Percent insectivorous fishes
8. Percent piscivorous fishes
9. Percent as introduced fish species

Table 5. Correlation coefficients of variables with values for the first two axes from the canonical correspondence analysis. Parentheses indicate cumulative percentage variation of site-environment relation explained by the CCA. Dashes are used instead of numbers when relationship is nonsignificant ($\alpha=0.05$).

Variable	CCA1 (23.2)	CCA2 (37)
Percent insectivorous fishes	0.731	-0.512
Epifaunal cover	0.676	0.397
Fish species richness	0.658	---
Number of darter species	0.635	---
Extent of Riparian zone	0.609	---
Stream Bank Vegetation	0.468	---
Percent stream slope	0.438	---
HBI	-0.448	---
Percent introduced fishes	-0.596	---
Percent tolerant fish species	-0.744	---
Ratio of entrenchment	---	0.501
Ratio of shredders to total taxa	---	0.490

Figure 9. First and second axes from the detrended canonical correspondence analysis of selected biotic metrics, habitat data and sites from 1st to 3rd order tributaries within the Upham Brook, Dragon Run, Polecat Creek and Totopotomoy Creek watersheds.



By examining the placement of sites in Figure 9 with the variables and their respective strengths (coefficients), we can describe the site conditions based on our physical and biotic parameters. Physically, the study sites symbolized in the lower right quadrant of Figure 9 are indicative of good quality instream epifaunal cover, quality riparian zone vegetation cover, and little to no stream entrenchment. From the macroinvertebrate data we find that those sites also possess low ratios of the shredder-feeding group (when compared with total taxa) and low overall HBI values. From the fish data the lower right sites have few introduced species and few species considered to be tolerant of various degraded conditions. Fish species richness should be highest at those sites and there should be good populations of insectivorous fishes and darter species present. Based on our knowledge and experience in watersheds of the coastal plains throughout the Atlantic seaboard, these characteristics represent those that would likely be associated with unimpaired streams characterized by high biotic integrity.

Model Development

We used stepwise linear regression to develop our reference stream model. Of the eighteen biotic metric and habitat variables (out of a total of 36 variables) from the final ordination analyses in the regression, three remained as statistically significant predictors of site placement (or grouping) following the stepwise procedure. Although still preliminary, the steps we have taken for this pilot study indicate that a coastal plain reference stream would have a sample score relating to the equation:

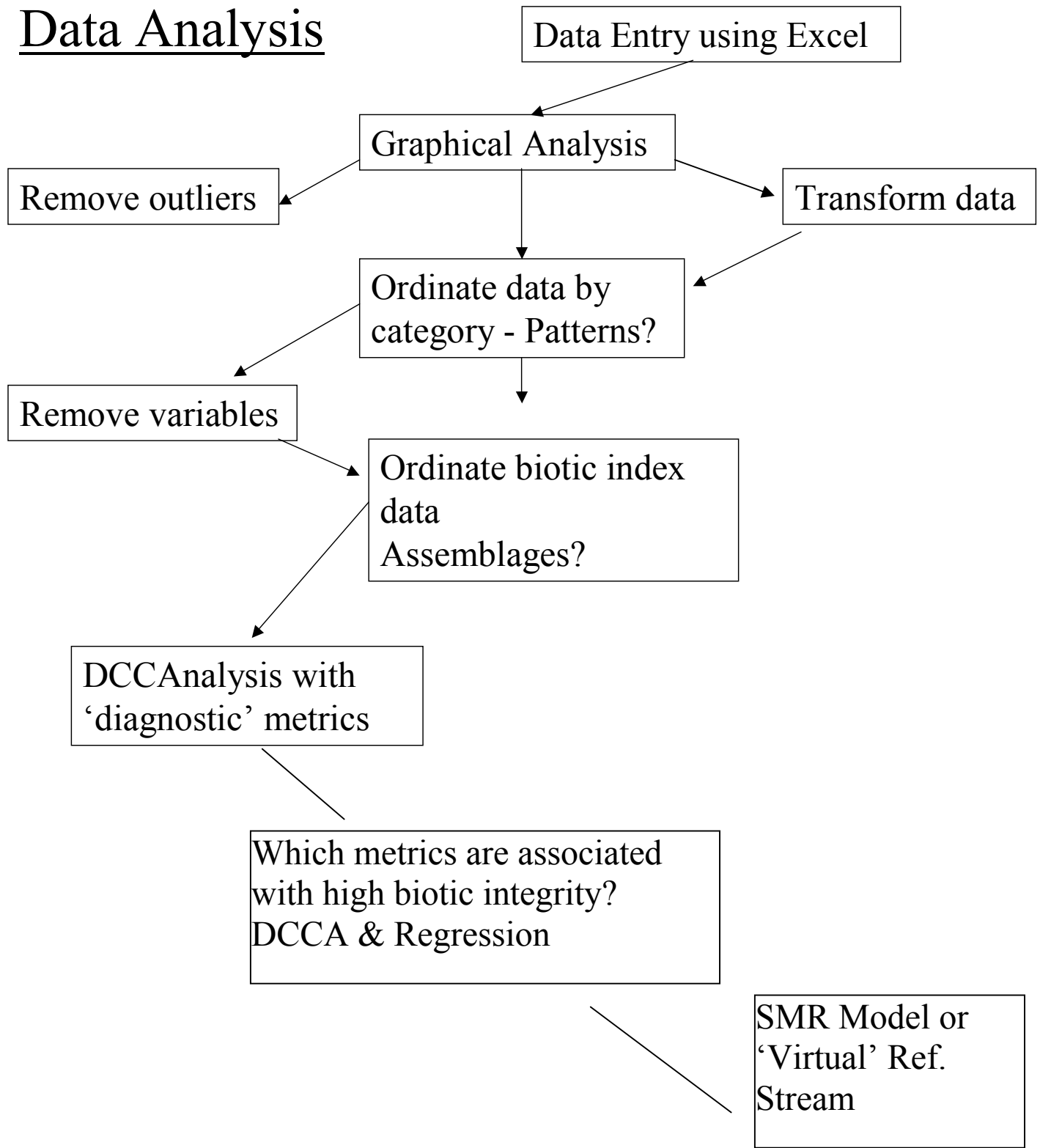
$$Y = -66.9 + 374.3 (\text{proportion of sample as insectivorous fishes}) + 92.10 (\text{proportion of quality epifaunal cover}) - 95.7 (\text{proportion of sample as tolerant fishes}).$$

According to this model, coastal plain reference streams should have a relatively high proportion of their fish community as insectivores and very low proportion as those species classified as tolerant of degraded conditions. In addition there will be a high degree of quality epifaunal cover. We do not suggest that this draft *virtual* model should be considered the working reference stream model for the Virginia Coastal Zone. Rather Phase I (this study) developed and tested a repeatable approach for developing model reference streams from empirical data and is the end result of a staged process of data collection, ordination, reduction, exploratory analyses, and final regression assessment. That this pilot study was successful in developing a statistically valid draft model of reference stream conditions supports the approach of Phase I and suggests that *virtual* reference streams could contribute substantially to stream assessment programs in Virginia. Access to more extensive data representing a wider array of Coastal Plain habitat types and disturbance regimes (Phase II & III) would serve to refine a series of virtual reference stream models, strengthen its precision, support model validation and allow development specific applications for stream monitoring, assessment, and protection.

Literature Cited

- Barbour, M.T. and J.B. Stribling. 1991. Use of habitat assessment in evaluating the biological integrity of stream communities. In: *Proceedings of Biological Criteria: Research and Regulation*. December, 1990. EPA/440-5-91-005. USEPA, Office of Science and Technology, Washington, D.C.
- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish*. 2nd edition. EPA 841-B-99-002. USEPA, Office of Science and Technology, Washington, D.C.
- Jenkins, R.E. and N.M. Burkhead. 1994. *Freshwater Fishes of Virginia*. American Fisheries Society. Bethesda, MD.
- Jongman, R.H.G., C.J.F. ter Braak and O.F.R. van Tongeren. 1987. *Data analysis in community and landscape ecology*. Pudoc, Wageningen.
- Karr, J.R. 1981. Assessment of biotic integrity using fish communities. *Fisheries* 6:21-27.
- Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. *Assessing biological integrity in running waters. A method and its rationale*. Ill. Nat. Hist. Survey Spec. Publication 5. Champaign.
- Lenat, D.R. 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. *Journal of North American Benthological Society*. 12:279-290.
- Maxted, J.R. M.T. Barbour, J. Gerritsen, V. Poretti, N. Primrose, A. Silvia, D. Penrose, and R. Renfrow. 1998. *Assessment framework for mid-Atlantic coastal plain streams using benthic macroinvertebrates*. Technical Rept. For USEPA, Region 3, Annapolis, MD.
- McIninch, S.P. and G.C. Garman 1999. *The anadromous clupeid fishes of the Chesapeake Bay. An evaluation of essential habitat and barriers to migration in the Rappahannock River basin*. Final Project Report to VA Dept of Game & Inland Fisheries, Richmond, VA.
- Pflakin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. *Rapid Bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish*. EPA/440-4-89-001. USEPA Office of Water, Washington, D.C.
- Rosgen, D. 1996. *Applied river morphology*. Wildlife hydrology, Pasoga Springs, CO.
- Smock, L., and G. Garman. 2001. *Polecat Creek Biomonitoring Study*. Annual Report to the Chesapeake Bay Local Assistance Department, Richmond, Virginia.
- Ter Braak, C.J. F. 1988. CANOCO – a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 3.12). Agricultural Mathematics Group, Wageningen.

Data Analysis



Appendix I Species codes and taxonomy for macroinvertebrates and fishes collected during pilot study.

Macroinvertebrates

Code	Order	Family	Genus	Species
AGGA	Amphipoda	Gammaridae	Gammarus	sp.
AHXX	Annelida	Hirudinea		
AOXX	Annelida	Oligochaetae		
BCCO	Bivalvia	Corbiculidae	Corbicula	fluminea
BITA			Bittacomorpha	sp.
BSMU	Bivalvia	Sphaeriidae	Musculium	sp.
BSPI	Bivalvia	Sphaeriidae	Pisidiumsp.	
BSSP	Bivalvia	Sphaeriidae	Sphaerium	sp.
CDHY	Coleoptera	Dytiscidae	Hydroporus	spp.
CEAN	Coleoptera	Elmidae	Ancyronyx	variegatus
CEDU	Coleoptera	Elmidae	Dubiraphia	spp.
CEMA	Coleoptera	Elmidae	Macronychus	glabratus
CEST	Coleoptera	Elmidae	Stenelmis	spp.
CGDI	Coleoptera	Gyrinidae	Dineutessp.	
CGGY	Coleoptera	Gyrinidae	Gyrinus spp.	
CHBE	Coleoptera	Hydrophilidae	Berosus sp.	
CHPE	Coleoptera	Haliplidae	Peltodytes	sp.
CNNO	Coleoptera			
CPAN	Coleoptera	Ptilodactylidae	Anchytarsus	bicolor
DCAX	Decapoda			
DCCL	Diptera	Culicidae	Culex	sp.
DCCU	Diptera	Ceratopogonidae	Culicoides	spp.
DCPA	Diptera	Ceratopogonidae	Palpomyia	spp.
DCPR	Diptera	Ceratopogonidae	Probezzia	sp.
DCXX	Diptera	Chironomidae		
DEMX	Diptera	Empididae	Hemerodromia	sp.
DEXX	Diptera	Ephydriidae		
DSXX	Diptera	Simuliidae		
DTHE	Diptera	Tipulidae	Hexatoma	spp.
DTPI	Diptera	Tipulidae	Pilaria	spp.
DTTA	Diptera	Tabanidae	Tabanus spp.	
DTTI	Diptera	Tipulidae	Tipula	abdominalis
EBBA	Ephemeroptera	Baetidae	Baetis	spp.
EBCA	Ephemeroptera	Baetidae	Callibaetis	spp.
ECCA	Ephemeroptera	Caenidae	Caenis	sp.
EEEP	Ephemeroptera	Ephemerellidae	Ephemerella	spp.
EEEU	Ephemeroptera	Ephemerellidae	Eurylophella	temporalis
EHSI	Ephemeroptera	Heptageniidae	Stenacron	sp.
EHST	Ephemeroptera	Heptageniidae	Stenonema	modestum
ELLE	Ephemeroptera	Leptophlebiidae	Leptophlebia	sp.
ELPA	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	sp.
ETTR	Ephemeroptera	Tricoridae	Tricorythodes	sp.
GAFE	Gastropoda	Ancylidae	Ferrissia sp.	
GHSO	Gastropoda	Hydrobiidae	Somatogyrus	spp.
GLLY	Gastropoda	Lymnaeidae	Lymnaea	sp.
GLPS	Gastropoda	Lymnaeidae	Pseudosuccinea	columnella
GPY	Gastropoda	Planorbidae	Gyraulus	spp.
GPHE	Gastropoda	Planorbidae	Helisoma	sp.
GPPH	Gastropoda	Physidae	Physa	sp.
GPPL	Gastropoda	Planorbidae	Planorbula	sp.
GPPS	Gastropoda		Physella sp.	

Macroinvertebrates

Code	Order	Family	Genus	Species
GVCA	Gastropoda	Vivparidae	Campeloma	sp.
HCTR	Hemiptera	Corixidae	Trichocorixa	sp.
HESP			Hesperocorixa	sp.
HXXX	Hydracarina			
IACA	Isopoda	Asellidae	Caecidotea	sp.
LXXX	Lepidoptera			
MCNI	Megaloptera	Corydalidae	Nigroniaserricornis	
MSSI	Megaloptera	Sialidae	Sialis	sp.
NXXX	Nematoda			
OABA	Odonata	Aeshnidae	Basiaeschna	sp.
OABO	Odonata	Aeshnidae	Boyeria	vinosa
OCAR	Odonata	Coenagrionidae	Argia	sp.
OCIS	Odonata	Coenagrionidae	Ischnura	sp.
OCCA	Odonata	Calopterygidae	Calopteryx	spp.
OCCO	Odonata	Cordulegastridae	Cordulegaster	sp.
OCEN	Odonata	Coenagrionidae	Enallagma	spp.
OCEP	Odonata	Corduliidae	Epithecasp.	
OCMA	Odonata	Corduliidae	Macromia	sp.
OGLA	Odonata	Gomphidae	Lanthus	sp.
OGGO	Odonata	Gomphidae	Gomphus	spp.
OGPR	Odonata	Gomphidae	Progomphus	obscurus
OGXX	Odonata	Gomphidae		
OLCI	Odonata	Libellulidae	Celithemis	sp.
OLER	Odonata	Libellulidae	Erythemis	sp.
OLLE	Odonata	Libellulidae	Leucorminia	
OLLI	Odonata	Libellulidae	Libellula	spp.
OLNA	Odonata	Libellulidae	Nannothemis	sp.
OLPA	Odonata	Libellulidae	Pachydiplax	longipenis
OLSO	Odonata	Libellulidae	Somatochlora	spp.
PCAL	Plecoptera	Capniidae	Allocaenia	sp.
PLLE	Plecoptera	Leuctridae	Leuctra	sp.
PNPR	Plecoptera	Nemouridae	Prostoia	sp.
PPCL	Plecoptera	Perlodidae	Clioperla	clio
PPEC	Plecoptera	Perlidae	Eccoptura	xanthenes
PPIM	Plecoptera	Perlodidae	Immature	
PTTA	Plecoptera	Taeniopterygidae	Taeniopteryx	spp.
TCAN	Trichoptera		Anisocentropus	sp.
TCHE	Trichoptera		Heteroplectron	sp.
THCH	Trichoptera	Hydropsychidae	Cheumatopsyche	spp.
THHD	Trichoptera	Hydroptilidae	Hydroptila	sp.
THIM	Trichoptera	Hydropsychidae	Immature	
THOX	Trichoptera	Hydroptilidae	Oxythira	sp.
TLLE	Trichoptera	Hydroptilidae	Stactobiella	sp.
TLNE	Trichoptera	Leptoceridae	Nectopsyche	sp.
TLOE	Trichoptera	Leptoceridae	Oecetis	sp.
TLOR	Trichoptera	Leptoceridae	Orthotrichia	sp.
TLPY	Trichoptera	Limnephilidae	Pycnopsyche	sp.
TMMO	Trichoptera	Molannidae	Molanna	blenda
TPCH	Trichoptera	Philopotamidae	Chimarra	sp.
TPDU	Turbellaria	Planariidae	Dugesia	tigrina
TPLY	Trichoptera		Lype	sp.
TPNY	Trichoptera	Polycentropodidae	Nyctiophylax	sp.

.Macroinvertebrates.

Code	Order	Family	Genus	Species
TPPO	Trichoptera	Polycentropodidae	Polycentropus	spp.
TPPT	Trichoptera	Phryganeidae	Ptilostomis	sp.
TPPU			Planaria	

Fishes

	Family	Genus/species	Common name
PMA	Petromyzontidae	Petromyzon marinus	Sea Lamprey
LAE	Petromyzontidae	Lampetra aepyptera	Least brook lamprey
ACA	Amiidae	Amia calva	Bowfin
ARO	Anguillidae	Anguilla rostrata	American eel
ENI	Esocidae	Esox niger	Chain pickerel
EAM	Esocidae	Esox americanus	Redfin pickerel
UPY	Umbridae	Umbra pygmaea	Eastern mudminnow
NCR	Cyprinidae	Notemigonus crysoleucas	Golden shiner
SCO	Cyprinidae	Semotilus corporalis	Fallfish
SAT	Cyprinidae	Semotilus atromaculatus	Creek chub
NLE	Cyprinidae	Nocomis leptcephalus	Bluehead chub
CYA	Cyprinidae	Cyprinella analostana	Satinfin shiner
LCO	Cyprinidae	Luxilus cornutus	Common shiner
NHU	Cyprinidae	Notropis hudsonius	Spottail shiner
NPR	Cyprinidae	Notropis procne	Swallowtail shiner
NCH	Cyprinidae	Notropis chalybaeus	Ironcolor shiner
HRE	Cyprinidae	Hybognathus regius	Eastern silvery minnow
EOB	Catostomidae	Erimyzon oblongus	Creek chubsucker
IPU	Ictaluridae	Ictalurus punctatus	Channel catfish
ANA	Ictaluridae	Ameiurus natalis	Yellow bullhead
ANE	Ictaluridae	Ameiurus nebulosus	Brown bullhead
NIN	Ictaluridae	Noturus insignis	Margined madtom
NGY	Ictaluridae	Noturus gyrinus	Tadpole madtom
ASY	Aphredoderidae	Aphredoderus sayanus	Pirate perch
GHO	Poeciliidae	Gambusia holbrooki	Eastern mosquitofish
APO	Centrarchidae	Acantharchus pomotis	Mud sunfish
CMA	Centrarchidae	Centrarchus macropterus	Flier
EBB	Centrarchidae	Enneacanthus obesus	Banded sunfish
EGL	Centrarchidae	Enneacanthus gloriosus	Bluespotted sunfish
MSA	Centrarchidae	Micropterus salmoides	Largemouth bass
LGU	Centrarchidae	Lepomis gulosus	Warmouth
LAU	Centrarchidae	Lepomis auritus	Redbreast sunfish
LMA	Centrarchidae	Lepomis macrochirus	Bluegill
LGI	Centrarchidae	Lepomis gibbosus	Pumpkinseed
PFL	Percidae	Perca flavescens	Yellow perch
PNO	Percidae	Percina notogramma	Stripeback darter
PPE	Percidae	Percina peltata	Shield darter
EOL	Percidae	Etheostoma olmstedii	Tessellated darter
EVI	Percidae	Etheostoma vitreum	Glassy darter

Appendix II

Physical habitat variables and respective symbols for ordination plots

EPIO – Epifaunal cover

PLS– Pool substrate

PLV – Pool variability

CHN – Channel alteration

SED – Sediment deposition

FLW – Channel flow status

BNKV – Bank Vegetative protection

BKST – Bank Stability

RIP – Riparian vegetative zone width

SLOPE – Rosgen slope parameter

SINUO – Sinuosity

RENT – Entrenchment ratio

RWD – Ratio stream width to depth